Cotton Catchment Communities CRC

SUMMER SCHOLARSHIP
Final Report

Part 1 - Summary Details

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Part 3 – Scholarship Report

1. Background:

Seedling establishment typically represents a significant bottleneck in the population dynamics of trees and shrubs in riparian and floodplain habitats of inland Australia (e.g. George et al., 2005; Thoms et al., 2007). Woody riparian species of semi-arid and arid regions often produce large numbers of seeds that typically germinate rapidly and opportunistically (e.g. Streng et al., 1989; Chong and Walker, 2005). The survival of seedlings in these hydrologically variable habitats, however, tends to be low and patchy, both spatially and temporally (Streng et al., 1989; Hughes, 1990; Cooper et al., 1999; Horton and Clark, 2001). Knowledge of the factors influencing seedling survival is therefore critical to understanding vegetation dynamics in these environments.

Vegetation structure in semi-arid and arid riparian and floodplain habitats is usually governed by the unpredictable patterns of flooding and drying that characterise these environments (e.g. Stromberg 2001; Brock et al., 2006). Hydrology also tends to have an overriding effect on seedling growth and survival in riparian habitats (e.g. Cooper et al., 1999; Horton and Clark, 2001; Capon et al., 2009). Seedlings are typically more vulnerable to the stresses imposed by flooding and drought than mature plants due to their smaller size (Cooper et al., 1999; Gindaba et al., 2004). Flooding can have a direct influence on seedlings through soil anoxia and toxicity, reduced light, mechanical damage and burial but may also provide structural support (Blom et al., 1990; 1996). The magnitude of these flood-induced effects are likely to vary with seedling size and developmental stage as well as flood characteristics such as depth, duration and timing (e.g. Capon et al., 2009). In arid systems, floodwaters also provide an important source of soil moisture during periods of flood recession which may favour seedling growth. The rate of drawdown of the water table can therefore be an important influence on the survival of seedlings into subsequent dry periods (Horton and Clark, 2011).

Woody species inhabiting riparian and floodplain habitats of arid and semi-arid regions display a range of traits and mechanisms that may facilitate regeneration under the unpredictable environmental conditions imposed by variable hydrologic regimes (e.g. Brock et al., 2006). These include growth variability and adaptation to local environmental conditions (Gibson et al., 1994), recruitment by large numbers of seedlings, rapid establishment and seedling growth (e.g. Capon et al., 2009) and rapid development of tap root systems which can track declining soil water (e.g. Kranjcec et al., 1998; Horton and Clark, 2001). The latter is a possible example of morphological plasticity which may be a beneficial trait in variable environments although is unlikely to evolve or be advantageous under the highly unpredictable conditions of arid floodplains (Capon et al., 2009).

Most research into seedling traits and factors influencing the growth and survival of seedlings of arid and semi-arid riparian and floodplain trees and shrubs has focused on species common to North America (e.g. Kranjcec et al., 1998; Cooper et al., 1999; Horton and Clark, 2001). Relatively few studies have investigated responses of Australian inland riparian tree or shrub seedlings to flooding and drought (Woolfrey and Ladd, 2001; Capon et al., 2009). Indeed, regeneration is poorly understood for many of the common tree and shrub species with wide distributions across Australia's vast inland floodplains (e.g. Roberts and Marston, 2011). In many of these regions, vegetation condition is declining in response to the alteration of flooding regimes associated with water resources developments and climate change (e.g. Thoms et al., 2007; Horner et al., 2009), and key tree populations often appear to be unable to sustain themselves (e.g. George et al., 2005). Consequently, there is an urgent need for improved information pertaining to the regeneration requirements of key species to inform water resources planning and management and better enable restoration and protection of these ecologically and culturally significant species.

This project investigated how flooding and drying are likely to influence the establishment of two woody riparian species of Australia's semi-arid cotton growing catchments of the northern Muarray-Darling Basin: *Acacia stenophylla* A.Cunn. exBenth. (river cooba) and *Casuarina cunninghamiana* Miq. (river she-oak; Plate 1). These species were selected due to their ecological significance (i.e. as important structural species), the paucity of published information on their regeneration and the availability of seed.

Both of the selected species are widely distributed across the Murray-Darling Basin with *A. stenophylla* dominating extensive woodland areas on floodplains of the western and northern Basin as well as occurring as an understorey to eucalypt forest and woodland in riparian areas, particularly along rivers of the northern Murray-Darling Basin (Roberts and Marston, 2011). In contrast, *C. cunninghamiana* is more frequently encountered in upland riparian zones on the western side of the Great Dividing Range, though not in Victoria, and tends to be limited to the more mesic eastern semi-arid lowlands of the Basin (Roberts and Marston, 2011). Conditions required for seedling establishment of *A. stenophylla* are poorly understood (Roberts and Marston, 2011). Large numbers of seedlings are frequently observed following floods although survival appears to be limited (Cunningham et al., 1991). More is known of regeneration of *C. cunninghamiana* (Roberts and Marston, 2011) and seedlings of this species appear to be favoured by short periods (i.e. < 5 days) of inundation (Woolfrey and Ladd, 2001). Studies of seedling tolerance of longer periods of submergence, however, have produced mixed results including complete mortality following two months of submergence in one study (Woolfrey and Ladd, 2001) versus initial growth and tolerance of complete submergence for up to 200 days in another (Evans 2003). Differences in seedling age may account for these apparently contradictory results (Roberts and Marston, 2011).

Studying seedling responses to flooding and drying in two species simultaneously provides the opportunity to contrast regeneration patterns and avoid findings which may appear to contradict with previous studies which have examined single species in isolation. The results may also be interpreted in light of differences in the distribution of the two species and provide a basis for predicting possible changes in these distributions in response to future conditions that may be imposed by climate change.

Plate 1. Photos of the study species: *Casuarina cunninghamiana (left) and Acacia stenophylla (right). Source: National Herbarium of NSW, www.plantnet.rbgsyd.nsw.gov.au*

2. Aims and Objectives:

This project used glasshouse experiments to compare seed germination patterns in the study species (i.e. *Acacia stenophylla* A.Cunn. exBenth. (river cooba) and *Casuarina cunninghamiana* Miq. (river she-oak) as well as seedling responses to broadly contrasting hydrologic conditions (i.e. submergence, water logging and drying) likely to be encountered by seedlings in their natural habitats. More specifically, this study addressed the following research questions:

1. How do germination patterns differ between the study species and which seed treatment produces the best germination results?
2. How do seedlings of the study species respond morphologically to flooding, waterlogging and drying?
3. How long can similarly aged seedlings of the study species survive under conditions of submergence and drought?
4. How do hydrologic conditions during early seedling life influence short-term responses to altered conditions?
5. Under what hydrologic conditions are the study species likely to successfully establish and how might this influence the distribution of these species?

It is anticipated that the findings of this project will contribute to water management planning in the northern Murray-Darling Basin by improving knowledge of watering requirements for regeneration of the study species as well as informing the design of ecological monitoring programs with respect to assessing information about patterns of seedling recruitment in terms of ecosystem 'health'.

This project addressed two key goals of the Cotton Catchment Communities CRC's Strategic Plan in relation to sustainable ecosystems and reducing catchment impacts: i) improving the assessment, management and monitoring of biodiversity and ecosystem service resources in cotton growing catchments, and ii) enabling decisions on natural resource management by growers and catchment bodies to be informed by science-based, best practice knowledge.

3. Methodology:

Seed collection and germination trial

Seeds of the two study species were collected in September 2011 at sites along the MacIntyre River in southern Queensland. Seeds were stored in paper bags and kept in dry conditions until the commencement of the germination trial. Prior to the germination trial, *A. stenophylla* seeds were manually removed from their casings and any clearly damaged or diseased seeds were discarded. *Casuarina cunninghamiana* seeds had collected in the bottom of sample bags and therefore required no manual extraction.

Seeds of both species were sown in seedling trays measuring 300 mm by 350 mm using Yates Co. seedling mix (Plate 2). Approximately 400 *C. cunninghamiana* seeds were sown directly with no treatment applied since seeds are known to germinate readily under warm, moist conditions (Boxshall and Jenkyn 2001). A range of treatments were applied to *A. stenophylla* seeds prior to sowing since scarification of seeds is used in the commercial propagation of this species (Roberts and Marston, 2011). Three treatments were used; 1.) no treatment on 60 seeds, 2.) light scarification with sandpaper on 60 seeds and 3.) boiling treatment on 200 seeds. The boiling treatment involved immersing seeds in 90 °C water for 60 seconds. Seeds then were cooled before sowing in the seedling mix. The sandpaper scarification method utilised fine grade sandpaper to lightly sand seeds in order to disturb the outer seed surface. Following sowing, seedling trays were watered twice daily to keep them moist and kept in a covered environment (Plate 2).

Plate 2. Photo of the germination trial set up.

Seedling establishment experiment

Following the germination trial, seedlings of the two study species were randomly selected for a seedlings establishment experiment. Forty *A. stenophylla* seedlings were selected but because of relatively low germination in *C. cunninghamiana*, only 21 seedlings were available for this species. All selected seedlings were removed from seedling trays and individually potted in 200 mm lengths of 50 mm diameter PVC pipe with 90 % shade cloth attached to the bottom to prevent the loss of sediment while allowing access to moisture. A thin layer of vermiculite was placed at the base of each tube followed by a mixture of low-grade coarse potting mix and potting sand at a ration of 10:1. Prior to re-potting, the total length, root length, stem length, number of leaves and number of stems was recorded for each seedling. For each *A.stenophylla* seedling the seed treatment was also recorded. Re-potted seedlings were left in the tubes for two days and watered daily to allow them to acclimatise before commencing flooding and drying treatments.

The seedling establishment experiment commenced on the 21st January, 2012 Selected plants were randomly allocated to one of four flooding treatments; 1.) full immersion, 2.) partial immersion (*A. stenophylla* only), 3.) flooding followed by drying and 4.) control. Each treatment, excluding partial immersion, was imposed on seven seedlings of *C. cunninghamiana* and ten *A. stenophylla* seedlings placed randomly within 40 L buckets. Each bucket contained between 8 and 9 individually potted seedlings as well as some empty tubes used to maintain seedlings in an upright position. The partial immersion treatment was only imposed on ten *A. stenophylla* due to the lack of sufficient *C. cunninghamiana* seedlings. Buckets were placed in a glasshouse at Griffith University in south-east Queensland and randomly placed along a bench, with the arrangement of the buckets rotated sporadically to accommodate for any influence of position, e.g. variation in light etc.. Control plants were kept moist throughout the experiment by watering daily as required. Water levels in the flooding and partial treatments were maintained at approximately 15 cm and 5 cm above the surface of the seedlings respectively. The flood then dry treatment was initially flooded to 15cm above the surface of the seedlings and then allowed to dry naturally over the experiment with no further water added.

A further ten *A. stenophylla* and five *C. cunninghamiana* seedlings were selected at random from the germination trial and their total length, root and stem length, number of leaves and number of stems recorded. These seedlings were then separated into root, leaf and stem material and dried at 104 °C until constant weight was obtained and their biomass measured.

Seedlings were non-destructively measured throughout the experiment at 15, 30, 40, 50, 55, 70, 80 and 90 days. At each of these times, plant height, number of leaves and number of stems were measured. After 90 days, a total of 16 *A. stenophylla* seedlings (comprising four from each treatment) and nine *C. cunninghamiana* seedlings (comprising two from the control treatment, three from the flood then dry treatment and four from the flooded treatment) were randomly selected, destructively harvested, separated into roots, shoots and leaves and dried to a constant weight to obtain biomass data.

The remaining seedlings were subjected to shifts in treatments to explore the effects of hydrologic conditions on early seedling growth on subsequent responses to flooding or drying. Amongst the 23 remaining *A. stenophylla* seedlings, two control plants were left to dry while two were kept moist. Of the seven remaining plants initially subjected to full immersion, three were left to dry and four kept moist. Of the six remaining seedlings initially subjected to partial immersion, three were allowed to dry and three were kept moist and of the six seedlings initially flooded then dried, three were allowed to continue to dry while three were returned to moist conditions. Only 12 *C. cunninghamiana* seedlings remained in the second phase of the experiment. Of these, there were four initial control seedlings which were all kept wet except one which was allowed to dry out. The four remaining seedlings initially subjected to full immersion were switched to the moist, control watering regime as were the four remaining seedlings initially flooded then dried. This second, exploratory phases of the experiment was conducted for 6 weeks to see how seedlings responded to the changed conditions at which time survival, final stem and root length as well as the number of leaves and stems were recorded.

Data analysis

Prior to analysis, all non-biomass measurements (i.e. stem height, root length, number of leaves and number of stems) were transformed as necessary (i.e. log, square root) after checking the assumptions of ANOVA with box plots and plots of model residuals. The experimental design was treated as a split-plot design, with watering treatment (fixed), bucket (random: 2 levels), and harvest time (fixed: 1 seedling harvested at days 0, 15, 30, 40, 50, 55, 70, 80 and 90). A partly nested full ANOVA model (Quinn and Keough, 2002) then was fitted including all terms plus appropriate interactions for all species and treatments, with the exception of the partially flooded *A. stenophylla* treatment where, due to logistic constraints, only a single bucket was used. Instead, a two factor ANOVA model was fitted for this treatment. If interaction term(s) were significant, appropriate one factor ANOVA models were then fitted for each level of the other factor(s), e.g. a significant treatment by time interaction resulted in models fitted for each time separately. As the number of *C.*

cunninghamiana stems was generally invariant for the first seven measuring times, we analysed this variable for the final two harvest times only. We used Tukey's tests to detect differences between treatment means after a significant main effect.

4. Results:

Germination

Acacia stenophylla seeds were the first seedlings to emerge in the germination trial, with the sandpaper treatment producing the first seedling to successfully emerge only 5 days after initial planting. Both the sandpaper and no treatment seeds germinated earlier than the heat treatment seeds. However, the heat treatment had the most success over a longer period with approximately 20 more seedlings present in heat treatment trays than within the sandpaper and no treatment trays (Plate 3). Within 10 days of planting, all *A.stenophylla* seed trays showed evidence of seedlings while the *Casuarina cunninghamiana* trays still had no seedlings present. *C. cunninghamiana* seedlings did begin to emerge after 14 days but at relatively low numbers. From the initial 300 seeds placed in the germination trays, only 55 *Casuarina* seedlings available for the next stage of the experiment.

Plate 3. Photo of *Acacia stenophylla* seedlings emerging from the germination trial

Seedling establishment

All of the seedlings remained alive at the end of the first phase of the seedling establishment experiment, including those subjected to 90 days of inundation. Considerable differences in growth responses was apparent however, both between the species as well as between treatments within each species.

A. stenophylla seedlings exhibited substantial variation in height over the first phase of the experiment with significant differences between the flooding then drying treatment and the flood treatment apparent from day 50 (Figure 1a, Tables 1 and 2). There was little or no difference in the mean height of *A. stenophylla* seedlings over time in the flood then drying or the partial flooding treatments but the control plants steadily increased in size throughout the experiment and were almost three times as tall as seedlings under the other treatments after 90 days (Figure 1a). *A. stenophylla* seedlings subjected to the full immersion flood treatment tended to show an initial decline in height but then maintained relatively constant heights from day 15 until day 90 (Figure 1a).

Trends in height were similar amongst *C. cunninghamiana* seedlings with control plants steadily increasing in size from day 15 (Figure 1b). Significant difference in mean height were detected amongst the treatments for *C. cunninghamiana* (Table 1), with the control group clearly differing from the two other treatments.). Although control *C. cunninghamiana* seedlings only reached a third of this size of control *A. stenophylla* seedlings, these were still approximately double the size of *C. cunninghamiana* seedlings subjected to the flooding and drying treatments (Figure 1b). *C. cunninghamiana* seedlings subjected to the flood then drying treatment also tended to be taller than seedlings under the flooding treatment (Figure 1b) although this was not statistically significant, probably due to the variability between replicates. *C. cunninghamiana* seedlings subjected to flooding also exhibited a reduction in height over the first 15 days of the experiment which then remained relatively constant until day 90 (Figure 1b).

Significant effects of treatment, time and an interaction between treatment and time were detected for the number of *A. stenophylla* leaves over the first phase of the experiment (Table 1, Figure 2b). *A. stenophylla* seedlings in the control treatments had significantly greater leaf numbers than those in other treatments from 30 days (Tukey HSD test $p< 0.05$; Table 2). No significant leaf loss was observed amongst flooded *A. stenophylla* seedlings and those subjected to the flood then drying treatment tended to acquire new leaves after day 55 (Figure 2b). In contrast, there appeared to be little effect of treatment on leaf number in *C. cunninghamiana* seedlings with most seedlings having only two leaves for the duration of the experiment (Figure 2a). As this variable was generally invariant no further analysis was performed.

There was a significant effect of treatment, time and the interaction between treatment and time on stem numbers amongst *A. stenophylla* seedlings (Table 1). Stem numbers in this species increased significantly in the control compared to other treatments after day 50 (Tukey HSD test p< 0.05; Table 2, Figure 3a). For the last two sampling times (80 and 90 days), stem numbers for *A. stenophylla* seedlings in the flooded then dry treatment were also significantly higher than those in the flooded treatment (Tukey HSD test p< 0.05; Table 2, Figure 3a). *C. cunninghamiana* seedlings in control treatments also had significantly greater numbers of stems compared with the other treatment (Table 1, Figure 3b).

A separate factorial ANOVA of the partial flooded treatment on *A. stenophylla* seedlings revealed no effect of time on any of the seedling attributes (i.e. leaf number, stem number or seedling height).

Root length and total seedling length in control seedlings of both species demonstrated significant increases over the 90 period of the first phase of the experiment (Figures 5 and 6). Seedlings subjected to all other treatments, however, exhibited relatively little change or, in the case of *C. cunninghamiana* seedling root length, a slight decline (Figures 5 and 6).

At the end of the first phase of the experiment, it was noted that two of the harvested *A. stenophylla* seedlings had developed a small number of nodules amongst the root fibres (Plate 4). Both of these plants had been under the control treatment and exhibited significant growth both in root length and overall. A further five *A. stenophylla* seedlings had developed adventitious roots, 1 from the control group and four from the partially flooded group (Plate 5). No evidence of adventitious roots was observed amongst any of the *C. cunninghamiana* seedlings.

At the time of writing, the biomass data had only just been processed and so we are only able to include preliminary analyses here (Table 3). These results indicate that significant differences in leaf and stem biomass occurred in *A. stenophylla* seedlings between treatments and were higher in control seedlings than those from all other treatments. In contrast, *C. cunninghamiana* seedlings had significantly higher root biomass from the flooded treatment.

Additionally, the second exploratory phase of the experiment had only recently concluded so presentation of detailed analyses here is not possible. Nevertheless, preliminary inspection of this data indicates that all *A. stenophylla* seedlings initially in the control treatment continued to grow and flourish over this six week period with significant increases in seedling height (four-fold), root length (three- to four-fold) and stem number (> three-fold), regardless of whether plants were kept moist or allowed to dry. Of those *A. stenophylla* seedlings initially subjected to full immersion, declines in leaf and stem number and seedling height occurred in plants left to dry as well as those kept moist. Additionally, all of the *C. cunninghamiana* seedlings that were continuously flooded in the first phase of the experiment, died over the subsequent six week period during which they were kept moist but not submerged.

Table 1. F values and significance levels of full ANOVA for main effects and interactions on leaf number, stem number and stem height.

	Acacia stenophylla			*Casuarina cunninghamiana*	
	Leaf number	Stem number	Stem height	Stem number	Stem height
Treatment1	288.3**	72.24*	154.1**	83.49*	95.8*
Time2	32.11***	34.68***	28.64***	2.483*	5.016***
Treatment*Time2	24.39***	17.78***	32.86***	1.3	6.639***

1 indicate error terms used in model as follows: 1: Bucket (Treatment) with 3 d.f., 2: Bucket(Treatment) * Time with 24 d.f., 3: Bucket(Treatment) * Time with 24 d.f..

* p≤0.05, ** p < 0.01, *** p≤0.001.

Table 2. *F* values and significance level for significant main effects from ANOVA of leaf number, stem number and stem height at each time interval separately. Error terms used in model as follows: 1: Bucket (Treatment) with 3 d.f.

	Acacia stenophylla			*Casuarina cunninghamiana*
	Leaf number	Stem number	Stem height	Stem height
0 days	0.439	0.439	0.298	1.205
15 days	2.753	0.538	1.246	1.052
30 days	14.554*	3.815	6.513	10.201*
40 days	22.027*	5.963	7.64	13.435*
50 days	37.867**	10.023*	13.741*	15.261*
55 days	48.270**	11.706*	24.909*	17.883*
70 days	84.03**	24.894*	48.637**	20.068*
80 days	92.574**	23.594*	55.016**	20.441*
90 days	91.060**	22.201*	67.521**	21.870*

* p≤0.05, ** p < 0.01, *** p≤0.001.

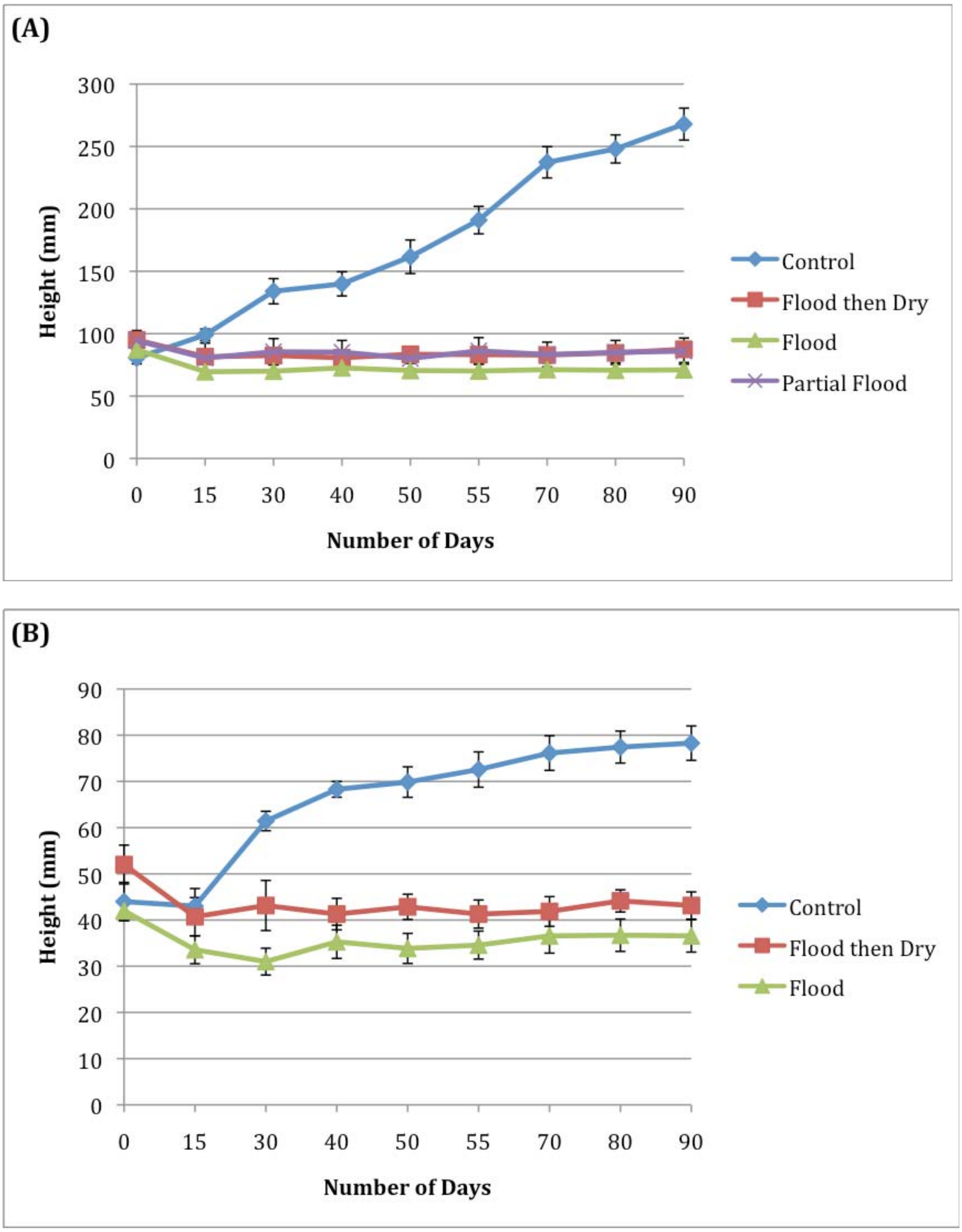

Figure 1. Change in mean seedling height for the first phase of the experiment for a) *Acacia stenophylla* seedlings (n=10 ± SE) and b) *Casuarina cunninghamiana* seedlings n=7 ±SE).

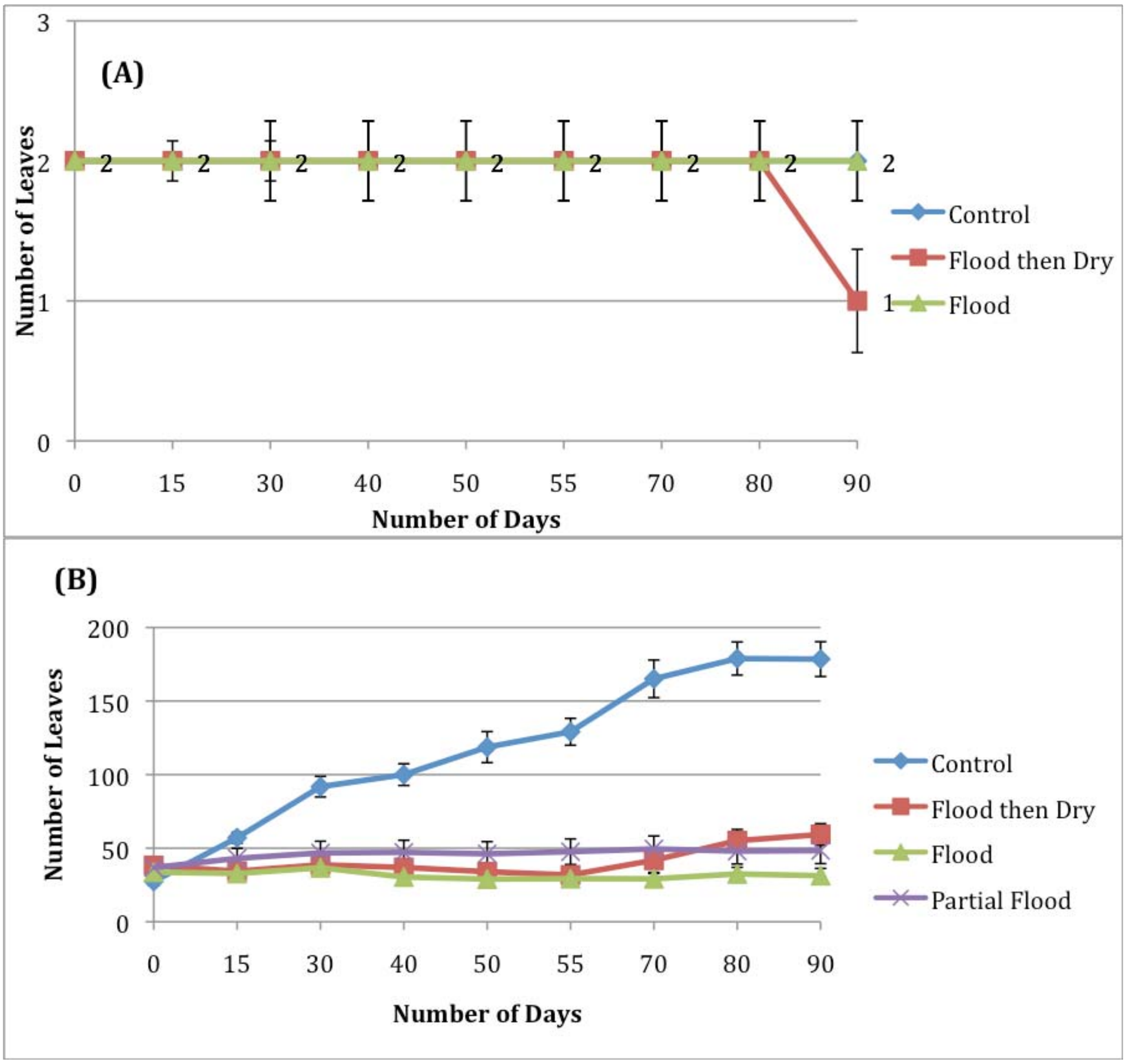

Figure 2. Variation in the mean number of leaves during the first phase of the experiment for a) *Casuarina cunninghamiana* seedlings (n=7 ±SE) and b) *Acacia stenophylla* seedlings (n=10 ± SE).

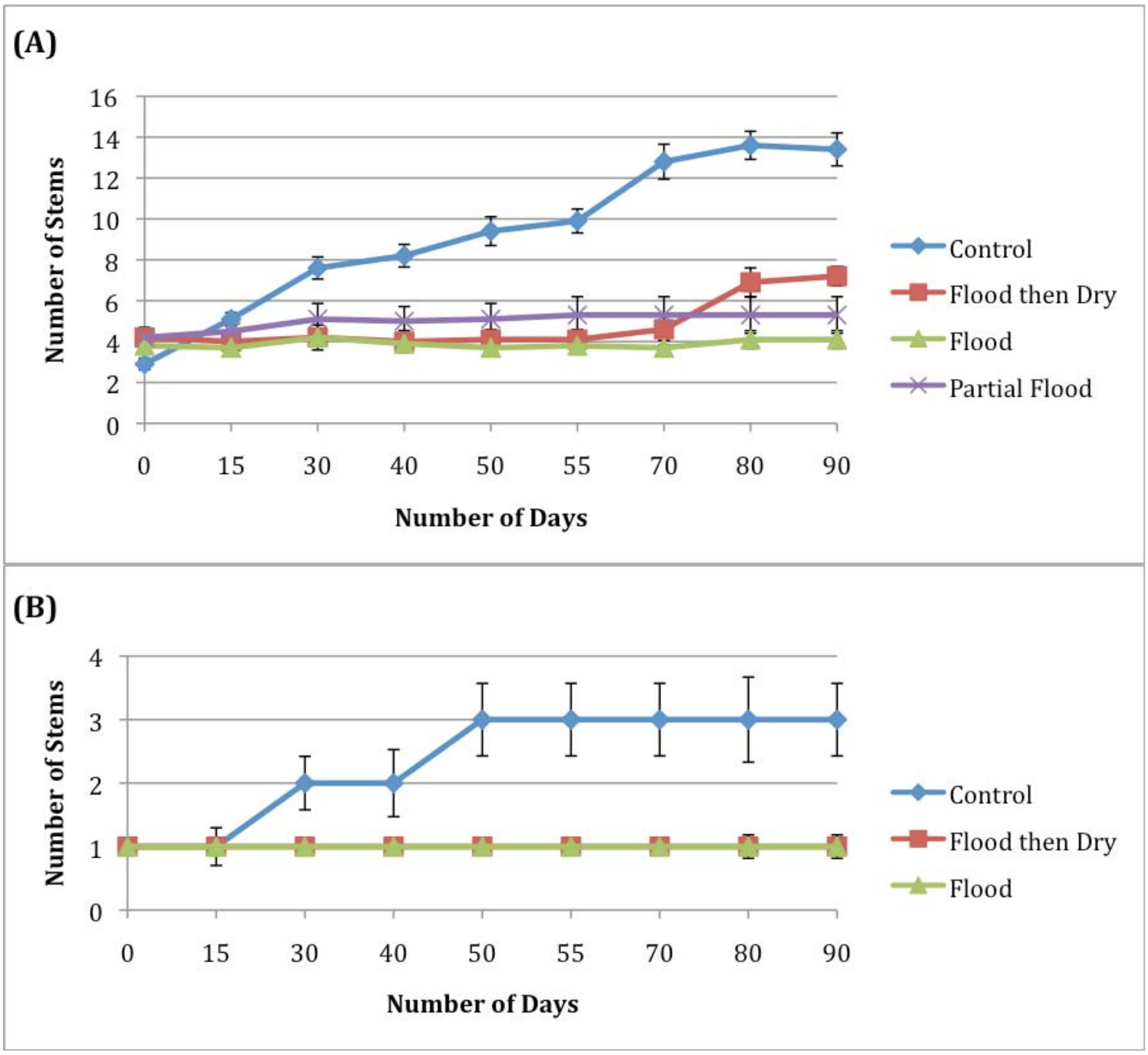

Figure 3. Variation in the mean number of stems during the first phase of the experiment for a) *Acacia stenophylla* seedlings (n=10 ± SE) and b) *Casuarina cunninghamiana* seedlings n=7 ±SE).

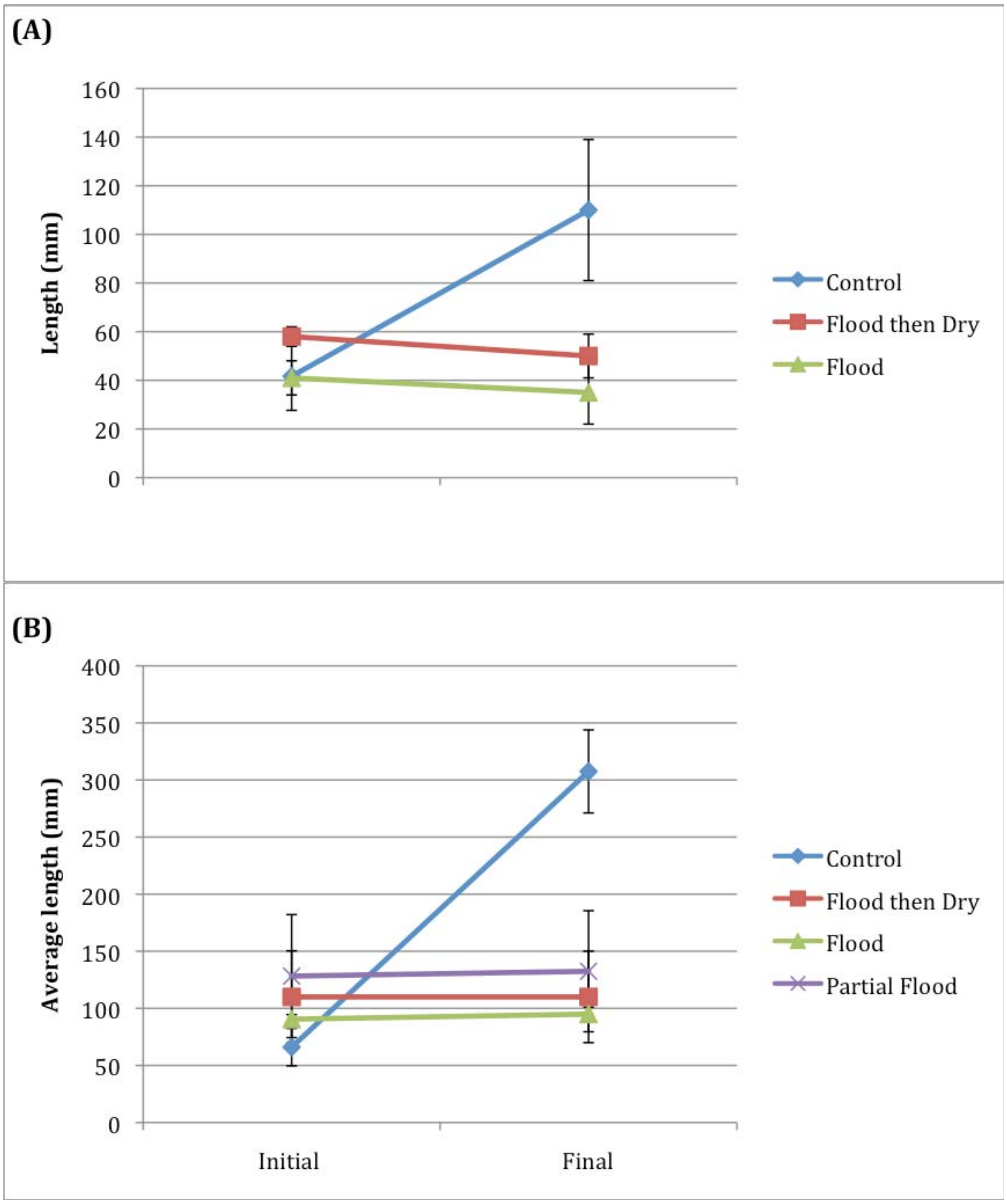

Figure 5. Changes in mean root length over the first phase of the experiment for a) *Casuarina cunninghamiana* seedlings (n=7 ±SE) and b) *Acacia stenophylla* seedlings (n=10 ± SE).

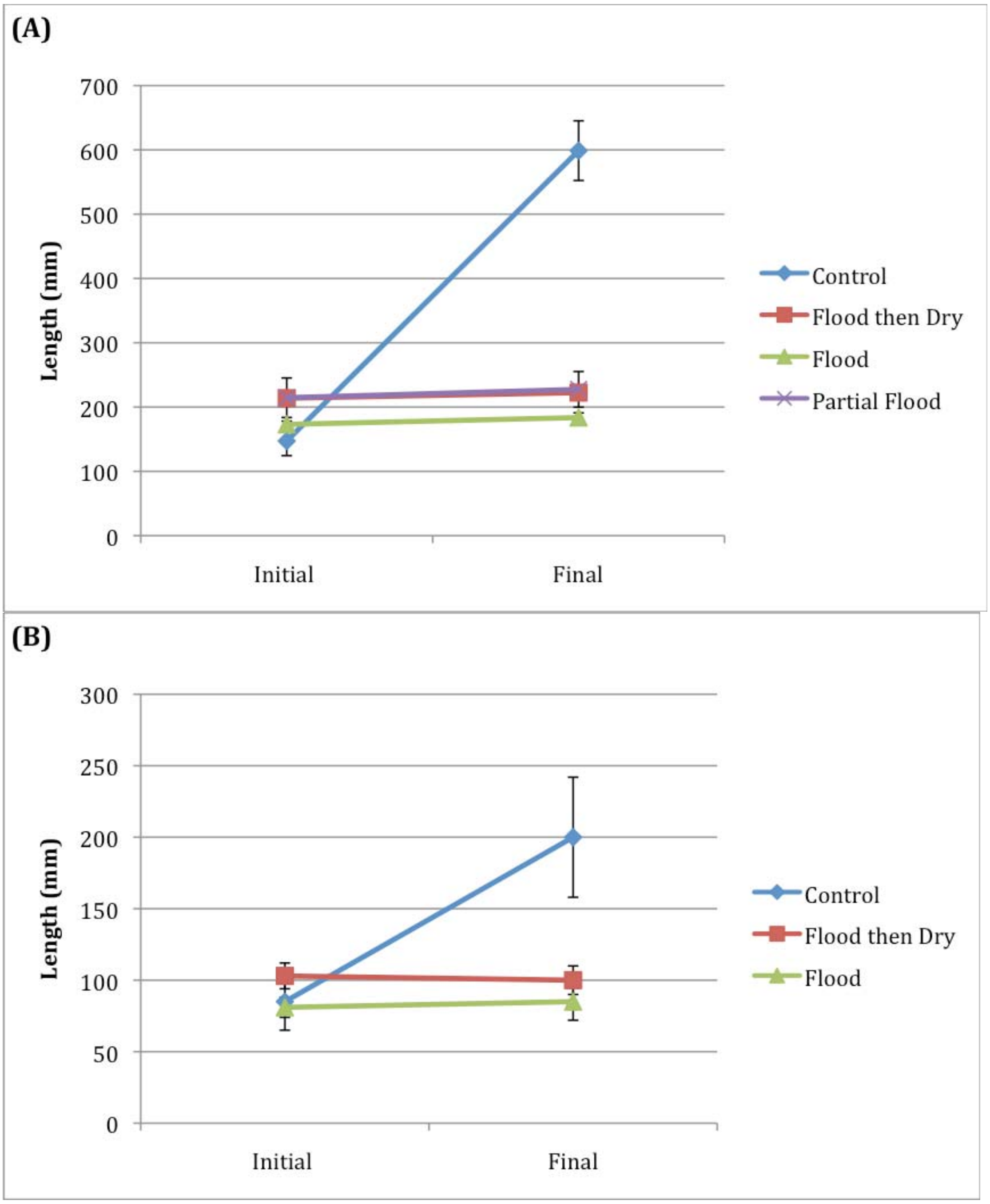

Figure 6. Changes in the mean total seedling length over the first phase of the experiment for a) *Acacia stenophylla* seedlings (n=10 ± SE) and b) *Casuarina cunninghamiana* seedlings n=7 ±SE).

Table 3. F values and significance levels of one-way ANOVA for treatment effects on root, leaf and stem biomass.

	Acacia stenophylla[1]	*Casuarina cunninghamiana*[2]
Root	0.938	6.397*
Leaf	5.023*	4.643
Stem	3.646*	1.216

1. d.f. = 3, : 2. d.f. = 2

* p≤0.05

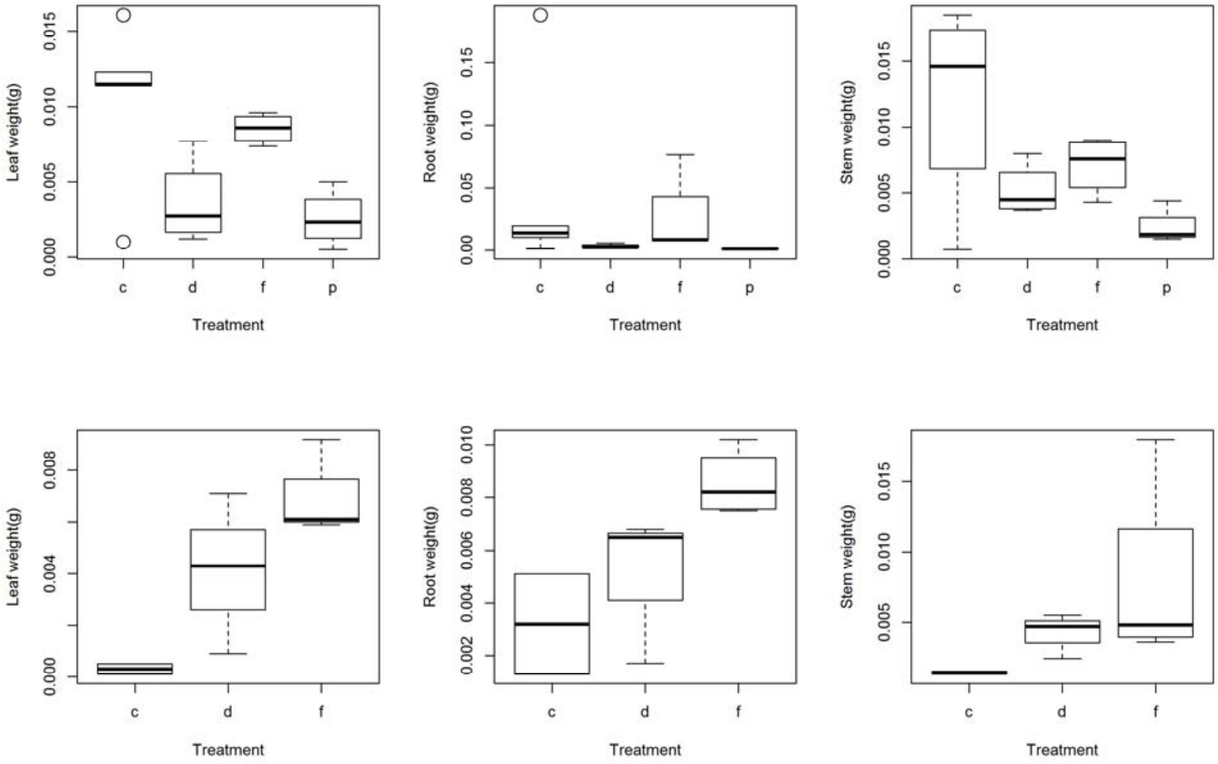

Figure 7. Differences in mean biomass amongst harvested seedlings at the end of the first phase of the experiment for top) *Acacia stenophylla* seedlings (n=10 ± SE) and bottom) *Casuarina cunninghamiana* seedlings n=7 ±SE). Graphs to the left show mean leaf biomass, graphs in the centre show mean root biomass and graphs to the right show mean stem weight.

Plate 4. An example of the nodules present on two of the *Acacia stenophylla* seedlings that were part of the control group. As evident by the photo on the right, the rootstock of the seedling was substantial and had developed amongst the root fibers a number of these nodules.

Plate 5. Of the *Acacia* seedlings harvested at the conclusion of the experiment 5 showed evidence of adventitious roots. The photo on the left shows an *Acacia stenophylla* seedling from the partial flooding trial, while the photo on the right is a close up of the stem showing evidence of adventitious roots.

5. Conclusion:

This study has demonstrated that young seedlings of both *Acacia stenophylla* and *Casuarina cunninghamiana* have considerable tolerance of a range of contrasting hydrologic conditions. Differences in regeneration responses are evident between these species however and these may contribute to the variation in their distribution and dominance in particular habitats.

Germination in both of the study species occurs readily in the absence of any seed treatment. *A. stenophylla* seeds, however, are likely to germinate more rapidly and at higher abundances than those of *C. cunninghamiana*. The relatively slow time to germination amongst *C. cunninghamiana* may mean emerging seedlings in this species have less time in which to become established in lowland floodplains during the favourable conditions in which sufficient soil moisture remains following floodwater recession. This could contribute to their relatively low frequency and extent in such habitats (e.g. Roberts and Marston, 2011). In contrast, the rapid emergence of *A. stenophylla* seedlings suggests that this species has the capacity to capitalise on similar periods of favourable soil moisture in these habitats.

Growth of *A. stenophylla* seedlings, in terms of seedling height and the acquisition of new leaves and stems, also appears to be more rapid than that of *C. cunninghamiana*, particularly under the moist conditions which are likely to be experienced following the recession of floodwaters. *A. stenophylla* seedlings under the moist control treatment used in this experiment grew over three times as large as *C. cunninghamiana* under similar conditions over the 90 day experimental period, also acquiring many more leaves and stems. Such rapid opportunistic growth is likely to favour seedling establishment of *A. stenophylla* in lowland floodplains following floodwater recession.

In both species, seedlings are clearly capable of surviving under total and partial submersion for considerable durations (i.e. up to 90 days) by conserving resources and producing little new growth, although initial declines in stem height may occur in response to inundation. Young seedlings of both species are also capable of persisting for a significant duration under dry conditions following floodwater recession and subsequent lowering of the water table but, as with flooded plants, are unlikely to accumulate biomass under such conditions.

The ability to persist under submergence could be advantageous for seedlings of species inhabiting hydrologically variable semi-arid floodplains provided plants are able to capitalise on favourable conditions should they subsequently occur. The preliminary findings of the second phase of this experiment suggest that *A. stenophylla* seedlings surviving 90 days of immersion are able to persist following floodwater recession under dry (i.e. rapid drawdown) or moist (i.e. slow drawdown) conditions. In contrast, seedlings of *C. cunninghamiana* that survive extended periods of submergence, appear to have a relatively limited capacity to survive following drawdown, even when soil moisture is maintained.

Survival of *A. stenophylla* seedlings during periods of soil waterlogging or partial submergence is likely to be at least partially facilitated by the development of adventitious roots. Adventitious roots may enable plants to cope with the soil anoxia associated with flooding but can also stabilise plants in the face of flood scour (Horton and Clark, 2001). The lack of adventitious roots on seedlings subjected to full immersion in this experiment may reflect the level of stress being experienced by these plants and the lack of resources available to allocate towards the accumulation of any biomass. Development of adventitious roots has also been observed on seedlings of *Muehlenbeckia florulenta* (tangled lignum), another common shrub species of Australian semi-arid lowland floodplains (Capon et al., 2009).

Overall, the findings of this study indicate that *A. stenophylla* seedlings are considerably more likely to successfully establish in lowland floodplain habitats of the northern Murray-Darling Basin than *C. cunninghamiana* seedlings. The slow germination rate and initial seedling growth of *C. cunninghamiana* suggests that emerging seedlings of this species in such habitats would experience favourable conditions following floodwater recession for significantly less time than *A. stenophylla* seedlings. Furthermore, *A. stenophylla* seedlings may be more likely to persist through additional flooding and return to a healthy condition following a second period of drawdown. *C. cunninghamiana* tends to be more dominant in upland riparian areas on the western side of the Great Dividing Range and previous studies have demonstrated that seedlings exhibit numerous traits that promote their establishment under conditions of hydraulic and substrate variability (e.g. Woolfrey and Ladd, 2001; Evans, 2003). The results of this study indicate that the limited extent of this species in the semi-arid lowland floodplains of the northern Murray-Darling Basin is also likely to be largely a result of its particular regeneration niche.

The results of this study have numerous implications for water resources management in the northern Murray-Darling Basin. Our results indicate that successful establishment of both *A. stenophylla* and *C. cunninghamiana* seedlings in riparian zones of lowland rivers in this region is likely to occur during the moist conditions following floodwater recession. Furthermore, seedling establishment in these species is probably favoured by slow drawdown of floodwaters, e.g. under conditions of low evaporation during Spring or Autumn, or by floodwater recession that is followed by periods of rainfall that can top up soil moisture. Changes to the frequency, extent, duration and timing of riparian and floodplain inundation may therefore be reflected by altered population dynamics in these important species. Our findings also indicate that regeneration of *C. cunninghamiana* in riparian zones of these lowland habitats may be particularly susceptible to changes in flooding regimes as a result of water resources development or climate change. Long-term monitoring of seedling growth and survival in this region is recommended to further understand regeneration responses of these species to hydrological change. Additional experimental work to further explore the effects of early seedling growth conditions on subsequent growth and survival is also warranted.

6. Highlights:

Given the limited knowledge available regarding the regeneration of *A. stenophylla* (e.g. Roberts and Marston, 2011), a highlight of this project has been establishing the capacity of seedlings of this species to survive under a range of hydrologic conditions, including continuous submergence for 90 days. The design of this experiment has also enabled a useful comparison of the two study species which contributes to an understanding of differences in the distribution and dominance of these species in different riparian habitats.

7. Presentations and public relations:

A draft manuscript for submission to a peer-reviewed international journal has been prepared to communicate the results of this study. It is also anticipated that the results of this project will be presented at the 2012 Australian Society for Limnology Conference to be held in Armidale, NSW in late November.

8. References:

Blom, C.W.P.M. and Voesenek, L.A.C.J., 1996. Flooding: the survival strategies of plants. *Trends in Ecology and Evolution* 11: 290–295.

Blom, C.W.P.M., Bogemann, G.M., Laan, P., van der Sman, A.J.M., van de Steeg, H.M. and Voesenek, L.A.C.J., 1990. Adaptation to flooding in plants from river areas. *Aquatic Botany* 38: 29–47.

Boxshall, B. and Jenkyn, T. 2001. River she-oak *Casuarina cuninghamiana*. Farm Forestry Species Profile for Northern Victoria (in Roberts and Marston, 2011).

Brock, M.A., Capon, S.J. and Porter, J.L., 2006. Disturbance of plant communities dependent on desert rivers. In: Kingsford, R. (Ed.), *Ecology of Desert Rivers.* Cambridge University Press, Cambridge, pp. 100–132.

Capon, S.J., James, C.S., William, L. and Quinn, G.P. 2009. Responses to flooding and drying in seedlings of a common Australian desert floodplain shrub: *Muehlenbeckia florulenta* Meisn. (tangled lingum). *Environmental and Experimental Botany* 66: 178-185.

Chong, C. and Walker, K.F., 2005. Does lignum rely on a soil seed bank? Germination and reproductive phenology of *Muehlenbeckia florulenta* (Polygonaceae). *Aust. J. Bot*. 53: 407–415.

Cooper, D.J., Merritt, D.M., Andersen, D.C. and Chimner, R.A., 1999. Factors controlling the establishment of Fremont cottonwood seedlings on the Upper Green River, USA. Regul. *Rivers: Res. Manage*. 15: 419–444.

Cunningham, G.M., Mulham, W.E., Milthorpe, P.L. and Leigh, J.H. 1991. *Plants of Western New South Wales*. Soil Conservation service of New South Wales.

Evans, L.J. 2003. *The influence of fluvial geomorphology on riparian vegetation in upland river valleys: south-eastern Australia.* Unpublished PhD thesis, University of Canberra (in Roberts and Marston, 2011).

George, A.K., Walker, K.F. and Lewis, M.M. 2005. Population status of eucalypt trees on the River Murray floodplain, South Australia. *River Research and Applications* 21: 271-282.

Gibson, A., E. P. Bachelard and K. T. Hubick. 1994. Growth strategies of *Eucalyptus camaldulensis* Dehnh at 3 sites in northern Australia. *Australian Journal of Plant Physiology* 21: 653-662.

Gindaba, J., Rozanov, A. and Negash, L. 2004. Response of seedlings of two Eucalyptus and three deciduous tree species from Ethiopia to severe water stress. *For. Ecol. Manage*. 201: 119–129.

Horner G.J., Baker P.J., Mac Nally R., Cunningham S.C., and Thomson J.R. 2009. Mortality of floodplain forests subjected to a drying climate and water extraction. *Global Change Biology* 15: 2176–2186.

Horton, J.L. and Clark, J.L. 2001. Water table decline alters growth and survival of *Salix gooddingii* and *Tamarix chinensis* seedlings. *For. Ecol. Manage.* 140: 239–247.

Hughes, F.M.R. 1990. The influence of flooding regimes on forest distribution and composition in the Tana River floodplain, Kenya. *J. Appl. Ecol.* 27: 475–491.

Kranjcec, J., Mahoney,W.M. and Rood, S.B. 1998. The responses of three riparian cottonwood species to water table decline. *For. Ecol. Manage*. 110: 77–87.

Quinn, G.P. and Keough, M.J., 2002. *Experimental Design and Data Analysis for Biologists.* Cambridge University Press, Cambridge.

Roberts, J. and Marston, F. 2011. *Water regime for wetland and floodplain plants: a source book for the Murray–Darling Basin*, National Water Commission, Canberra.

Streng, D.R., Glizenstein, J.S. and Harcombe, P.A., 1989. Woody seedling dynamics in an East Texas floodplain forest. *Ecol. Monogr*. 59: 177–204.

Stromberg, J.C., 2001. Restoration of riparian vegetation in the south-western United States: importance of flow regimes and fluvial dynamism. *J. Arid Environ*. 49: 17–34.

Thoms, M., Capon, S., James, C., Padgham, M. and Rayburg, S. 2007. *Narran Ecosystem Project: the response of a terminal wetland system to variable wetting and drying.* Murray–Darling Basin Commission, Canberra.

Woolfrey, A.G. and Ladd, P.G. 2001. Habitat preference and reproductive traits of a major Australian riparian tree species (*Casuarina cunninghamiana*). *Australian Journal of Botany* 49: 705-715.

9. Executive Summary:

This project investigated regeneration traits of two important woody species of riparian zones in the northern Murray-Darling Basin: *Acacia stenophylla* A.Cunn. exBenth. (river cooba) and *Casuarina cunninghamiana* Miq. (river she-oak). Seeds collected from sites along the MacIntyre River were used for a germination trial to compare germination rates between species and seed treatments. Seedlings emerging from this germination trial were then used in a glasshouse experiment to determine seedling growth responses to a range of treatments including moist conditions, partial submergence, total submergence and flooding followed by drying over a 90 day period.

Germination was rapid (from 5 days) for *Acacia stenophylla* seeds and abundant but for *Casuarina cunninghamiana* did not commence for 2 weeks with considerably lower percentage of seeds germinating. Seedlings of both species exhibited considerable tolerance of the range of conditions imposed and no seedlings died during this experimental period. In both species, flooding resulted in initial declines in seedling growth (up to 15 days) after which seedlings survived but exhibited little growth or accumulation of new leaves or stems. Seedlings allowed to dry following initial flooding also displayed a similar holding pattern. In contrast, seedlings subjected to moist, well-watered conditions exhibited significant opportunistic growth during the study period, particularly *Acacia stenophylla* seedlings which grew three times larger than *Casuarina cunninghamiana* seedlings under the same conditions and accumulated many more stems and leaves.

Overall, the findings of this study indicate that *A. stenophylla* seedlings are considerably more likely to successfully establish in lowland floodplain habitats of the northern Murray-Darling Basin than *C. cunninghamiana* seedlings. The slow germination rate and initial seedling growth of *C. cunninghamiana* suggests that emerging seedlings of this species in such habitats would experience favourable conditions following floodwater recession for significantly less time than *A. stenophylla* seedlings. As a result, regeneration of *C. cunninghamiana* in these habitats is likely to be more vulnerable than *A. stenophylla* to changes in flooding regimes due to water resources development and climate change.